

Cotransfection of 293Cre cells with pBHG10lox and a "Lox" shuttle plasmid for generation of Ad expression vectors

09973464-103604

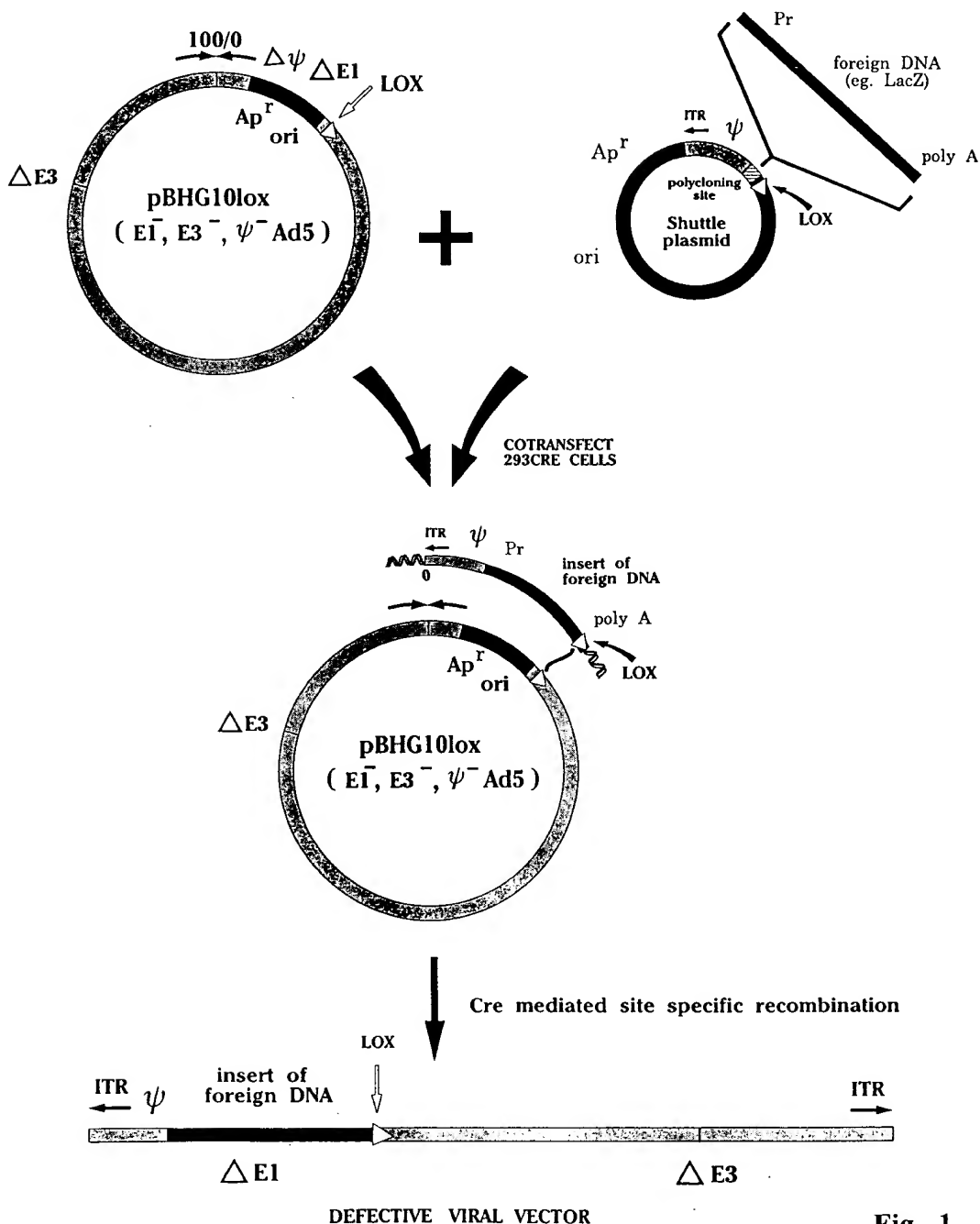


Fig. 1

Cotransfection of 293Cre cells with pBHG10lox and a "lox" shuttle plasmid for generation of Ad expression vectors

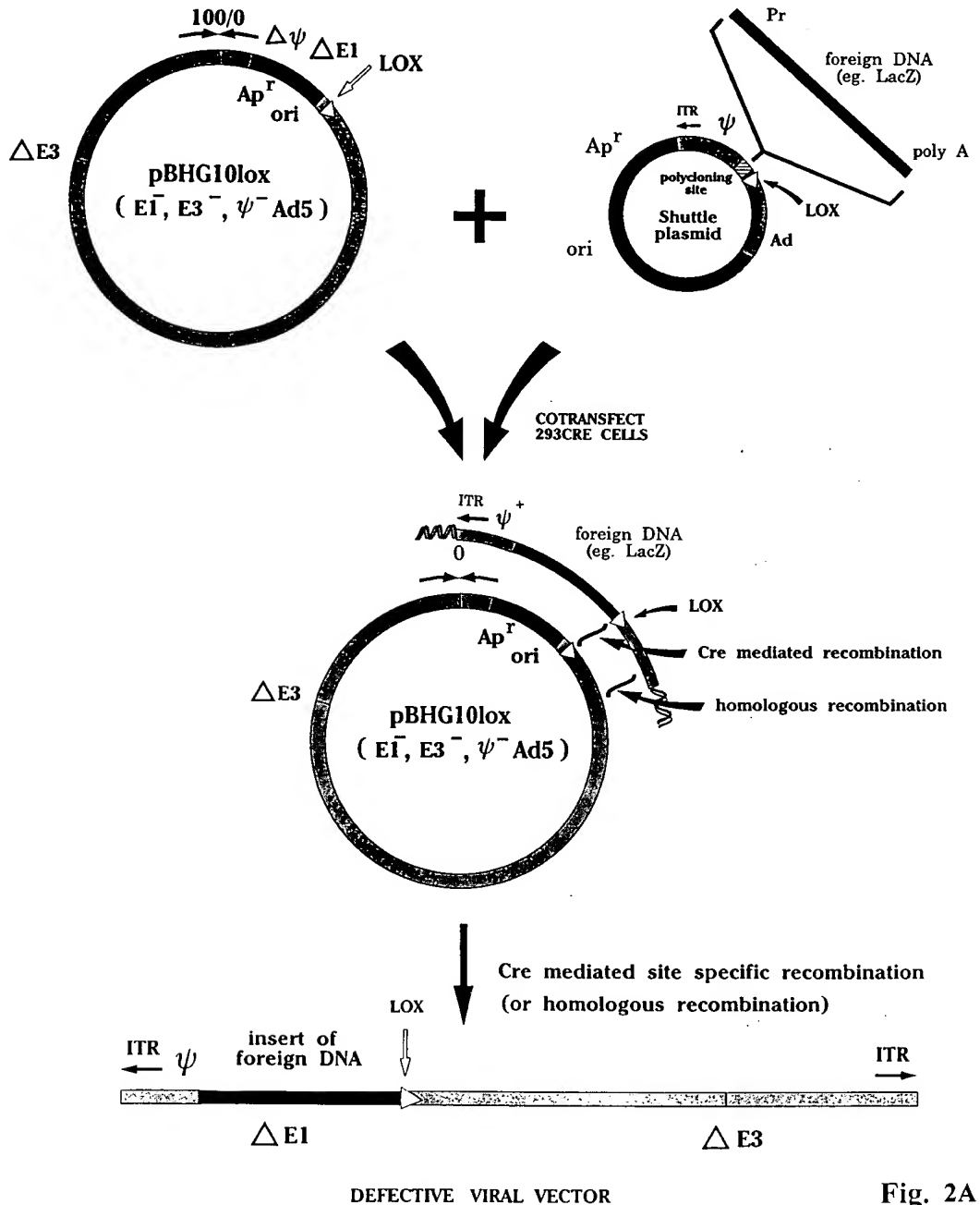


Fig. 2A

CONSTRUCTION OF VARIOUS SHUTTLE PLASMIDS

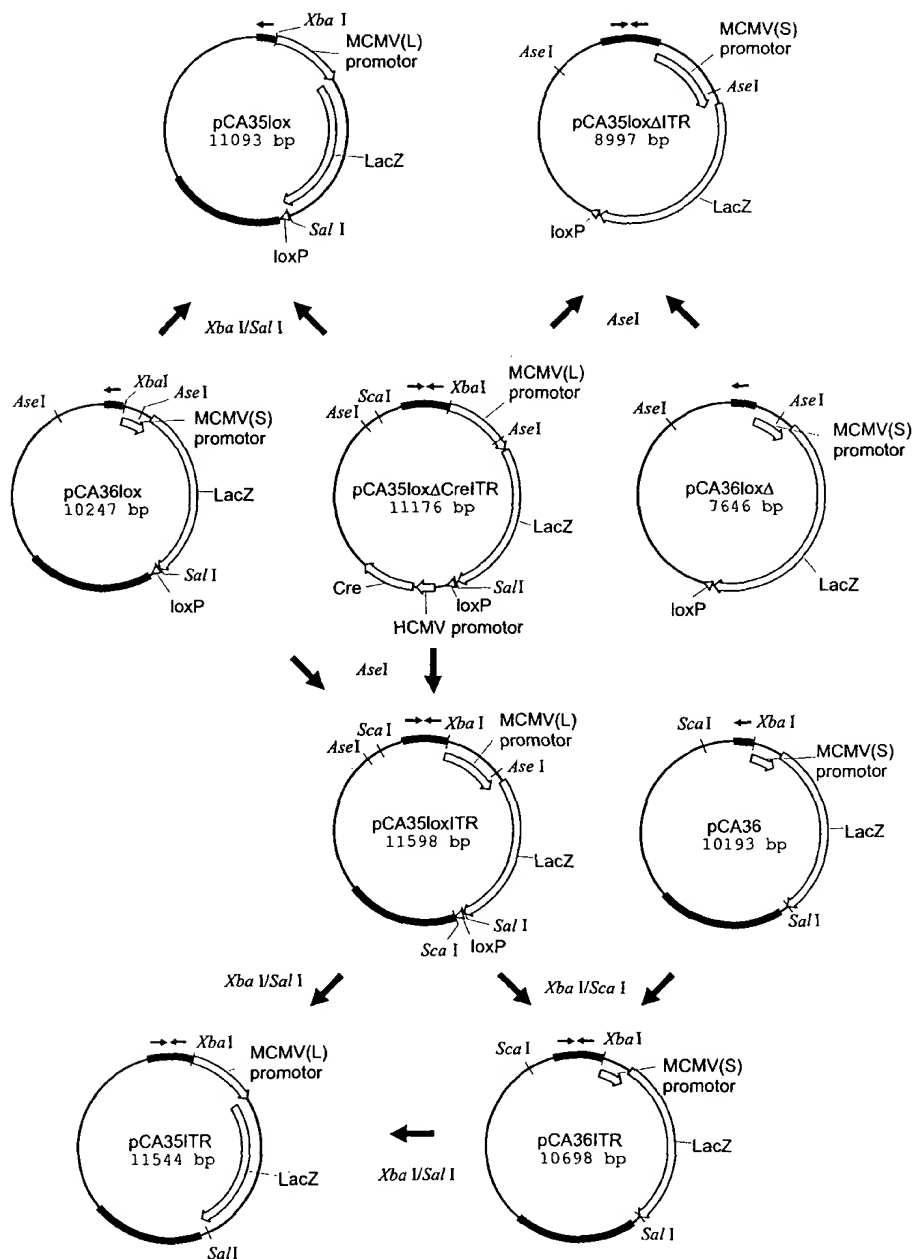


Figure 2B

OLIGONUCLEOTIDES USED IN CLONING

AB3233/3234 : loxP linker

SEQ. ID. NO. : 1

loxP site

*Bam*H I/*Bgl* II
overhang

5' GATCCAATAACTTCGTATAGCATACATTATACGAAGTTATAAGTACTGAATTCG 3'
3' GTTATTGAAGCATATCGTATGTAATATGCTTCAATATTCATGACTTAAGCCTAG 5'

*Bam*H I/*Bgl* II
overhang

SEQ. ID. NO. : 2

AB14626/14627 : Multiple Cloning Site

SEQ. ID. NO. : 3

Sma I *Bgl* II *Hind* III *Sac* I

Sal I overhang

5' AATTCCCCGGGAGATCTAAGCTTGAGCTCG 3'
3' GGGGCCCTCTAGATTCGAACTCGAGCAGCT 5'

*Eco*R I overhang

SEQ. ID. NO. : 4

AB6920/6921 : loxP linker

SEQ. ID. NO. : 5

Cla I

Xba I overhang

5' CTAGCAATAACTTCGTATAGCATACATTATACGAAGTTATATCGATG 3'
3' GTTATTGAAGCATATCGTATGTAATATGCTTCAATATAGCTACGATC 5'

Xba I overhang

SEQ. ID. NO. : 6

AB14680/14681 : loxP linker

SEQ. ID. NO. : 7

Cla I

Blp I overhang

5' TGACAATAACTTCGTATAGCATACATTATACGAAGTTATATCGATG 3'
3' GTTATTGAAGCATATCGTATGTAATATGCTTCAATATAGCTACACT 5'

Blp I overhang

SEQ. ID. NO. : 8

Fig. 3

CONSTRUCTION OF A CIRCULAR GENOMIC PLASMID FOR Ad VECTOR RESCUE USING THE Cre/ loxP SYSTEM

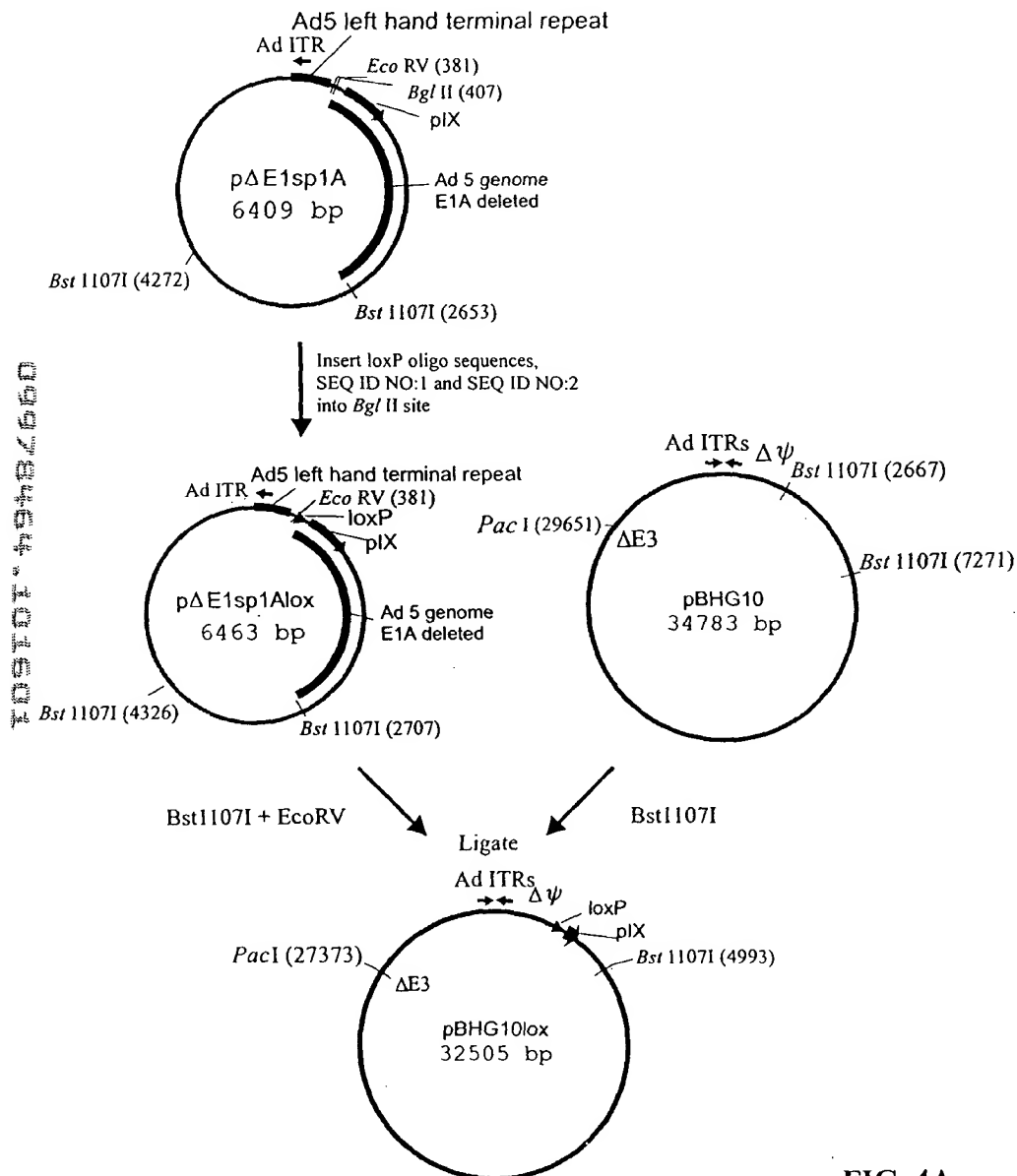


FIG. 4A

FIG. 4B-1 CONSTRUCTION OF pBHGdX1Plox

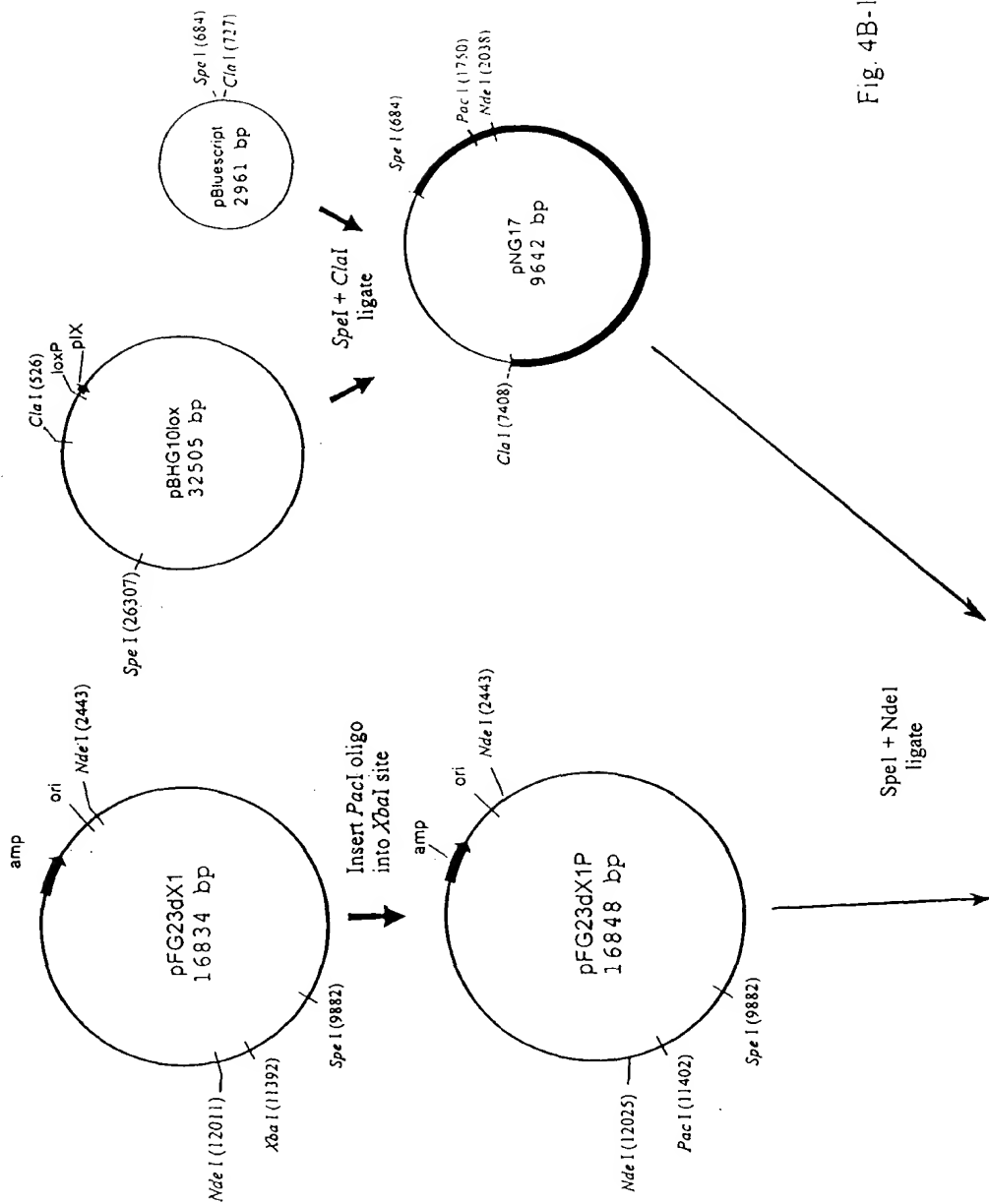
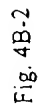


Fig. 4B-1

CONSTRUCTION OF pBHGdX1Plox



CONSTRUCTION OF pBHGE3lox

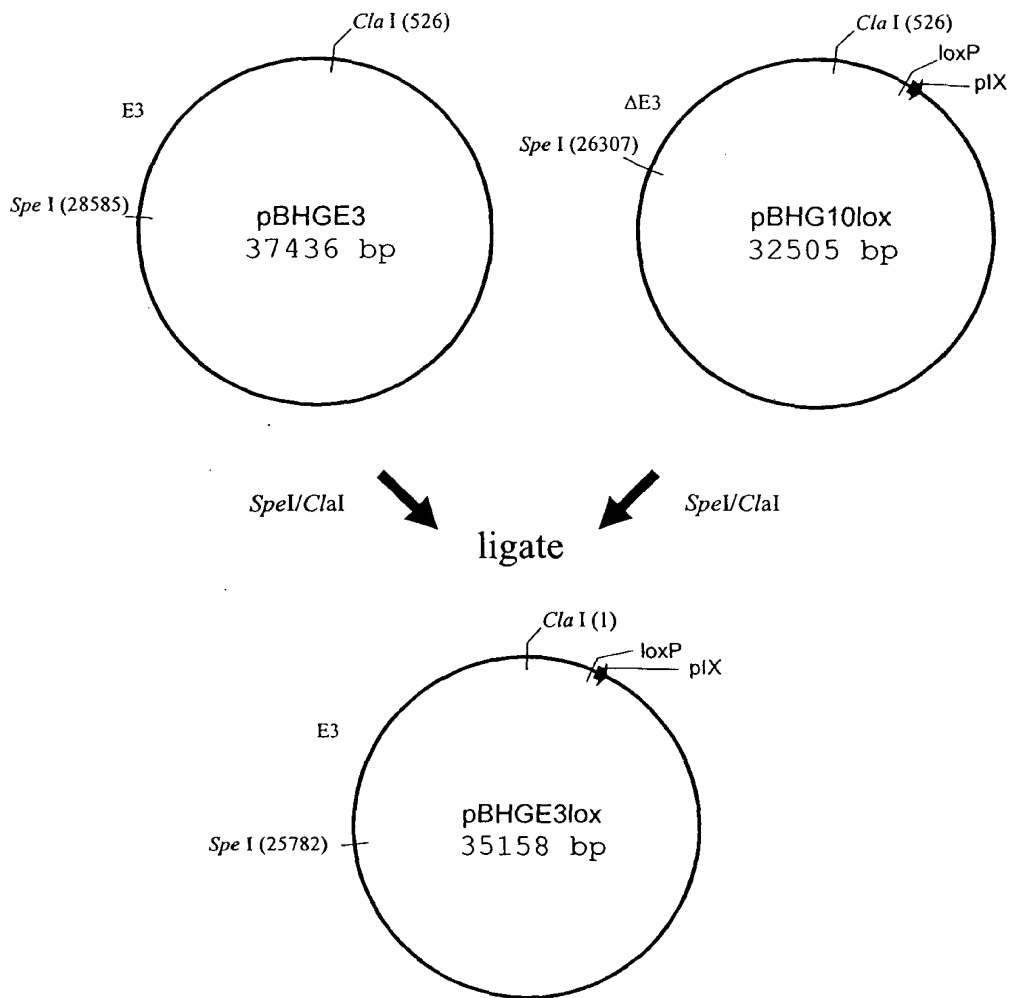


Fig. 4C

CONSTRUCTION OF Ad GENOMIC PLASMIDS ENCODING CRE

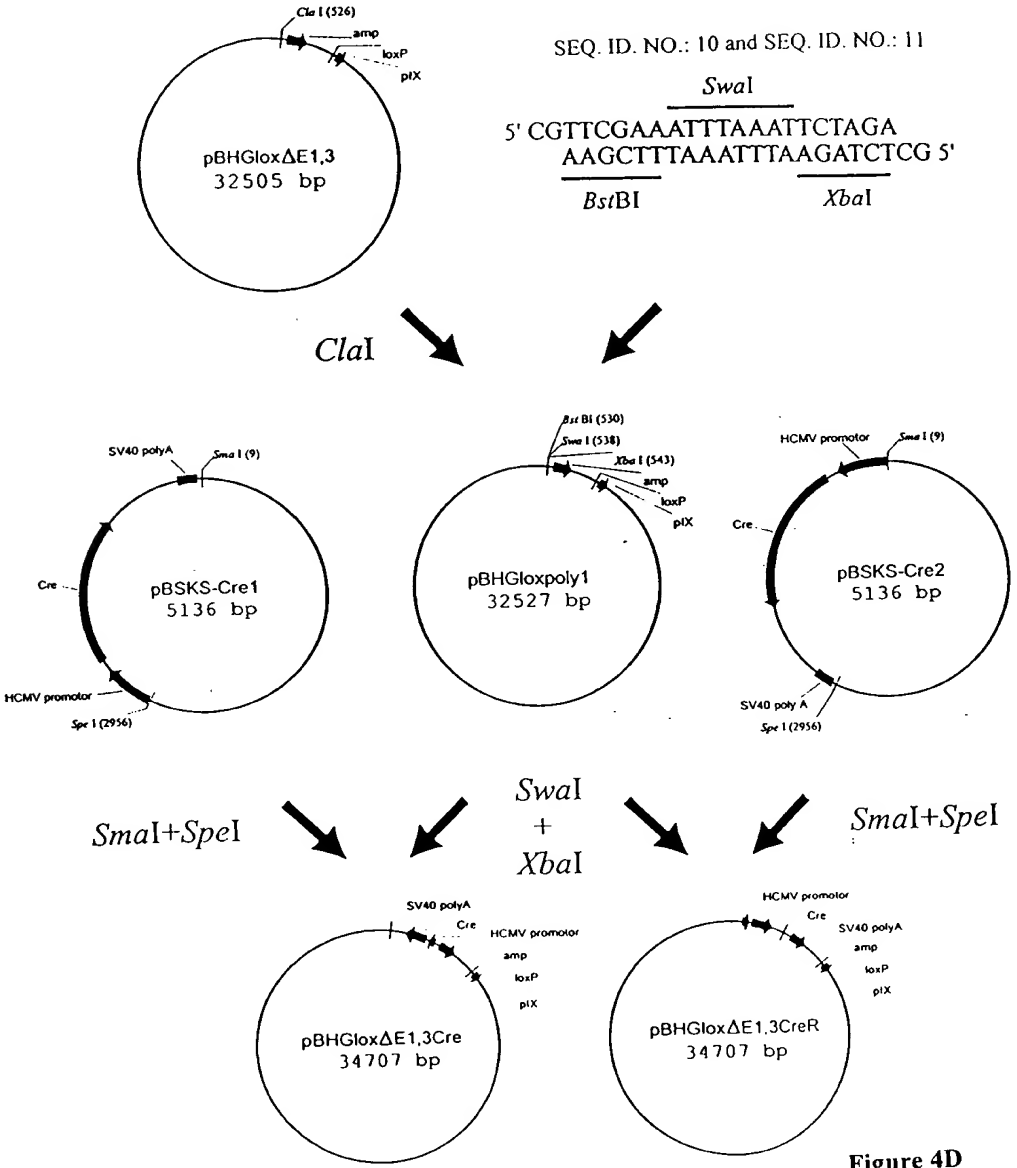
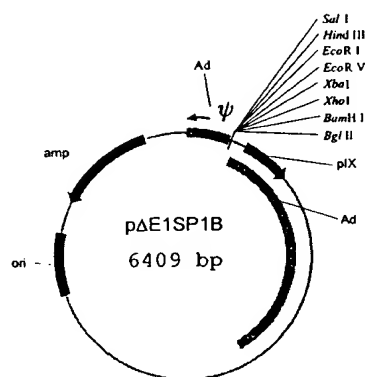
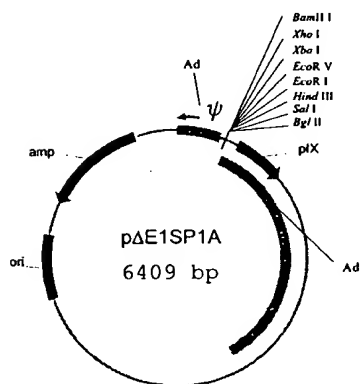
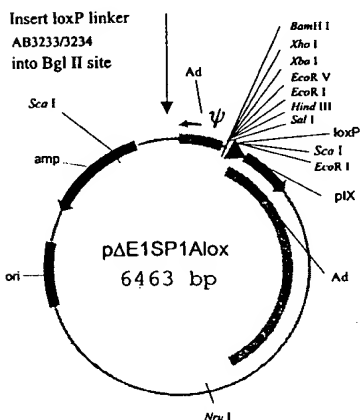


Figure 4D

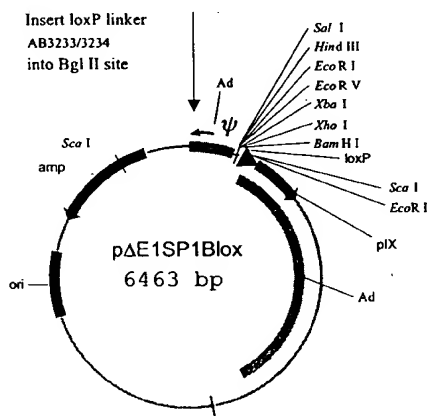
CONSTRUCTION OF pΔE1SP1A & pΔE1SP1B loxP PLASMIDS FOR RESCUE OF FOREIGN DNA



Insert loxP linker
AB3233/3234
into Bgl II site

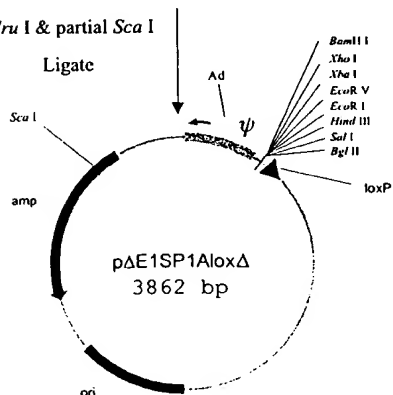


Insert loxP linker
AB3233/3234
into Bgl II site



Nru I & partial Sca I

Ligate



Nru I & partial Sca I

Ligate

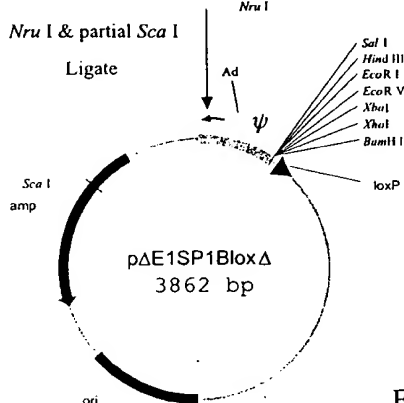


Fig. 5A

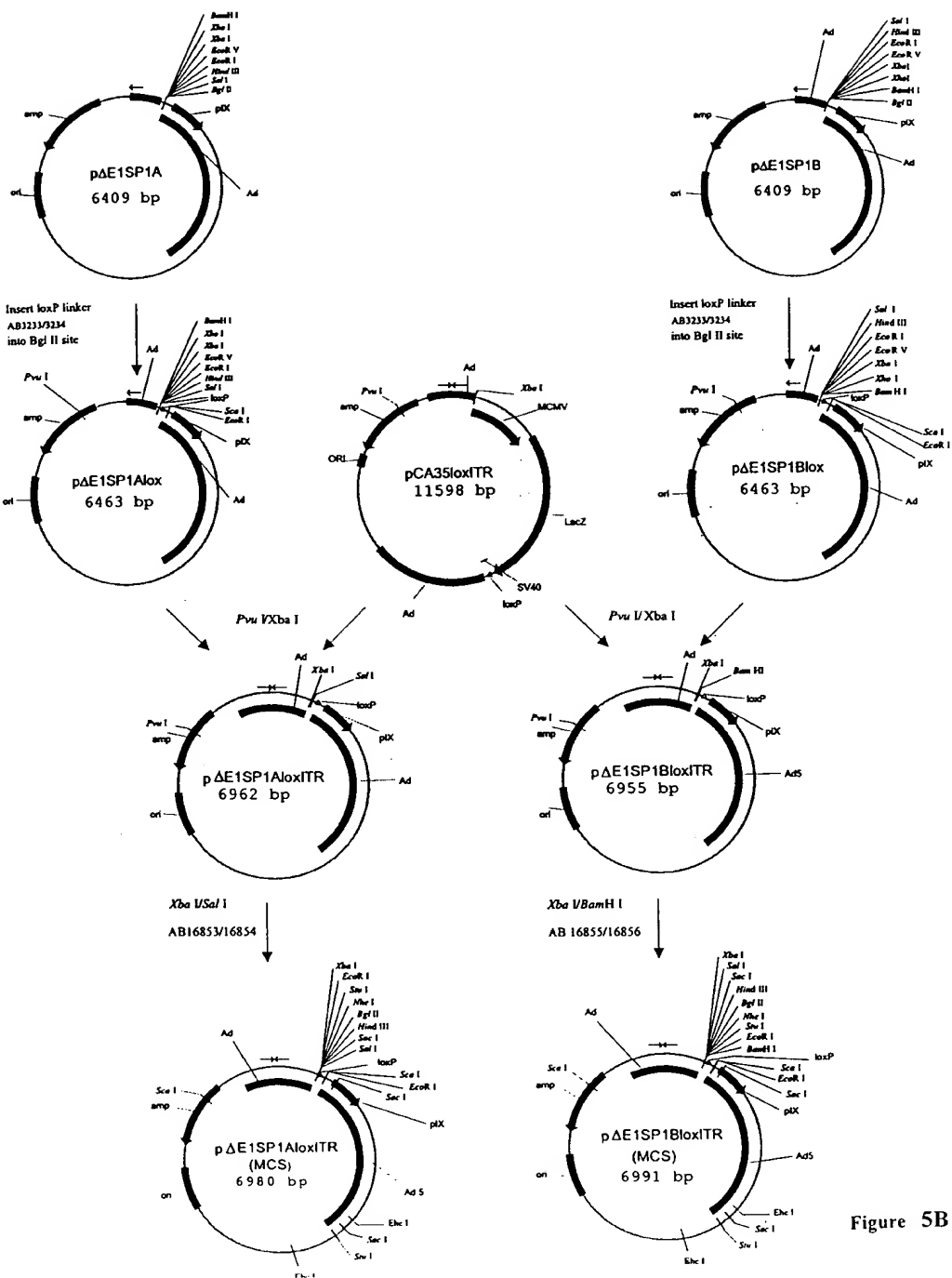


Figure 5B

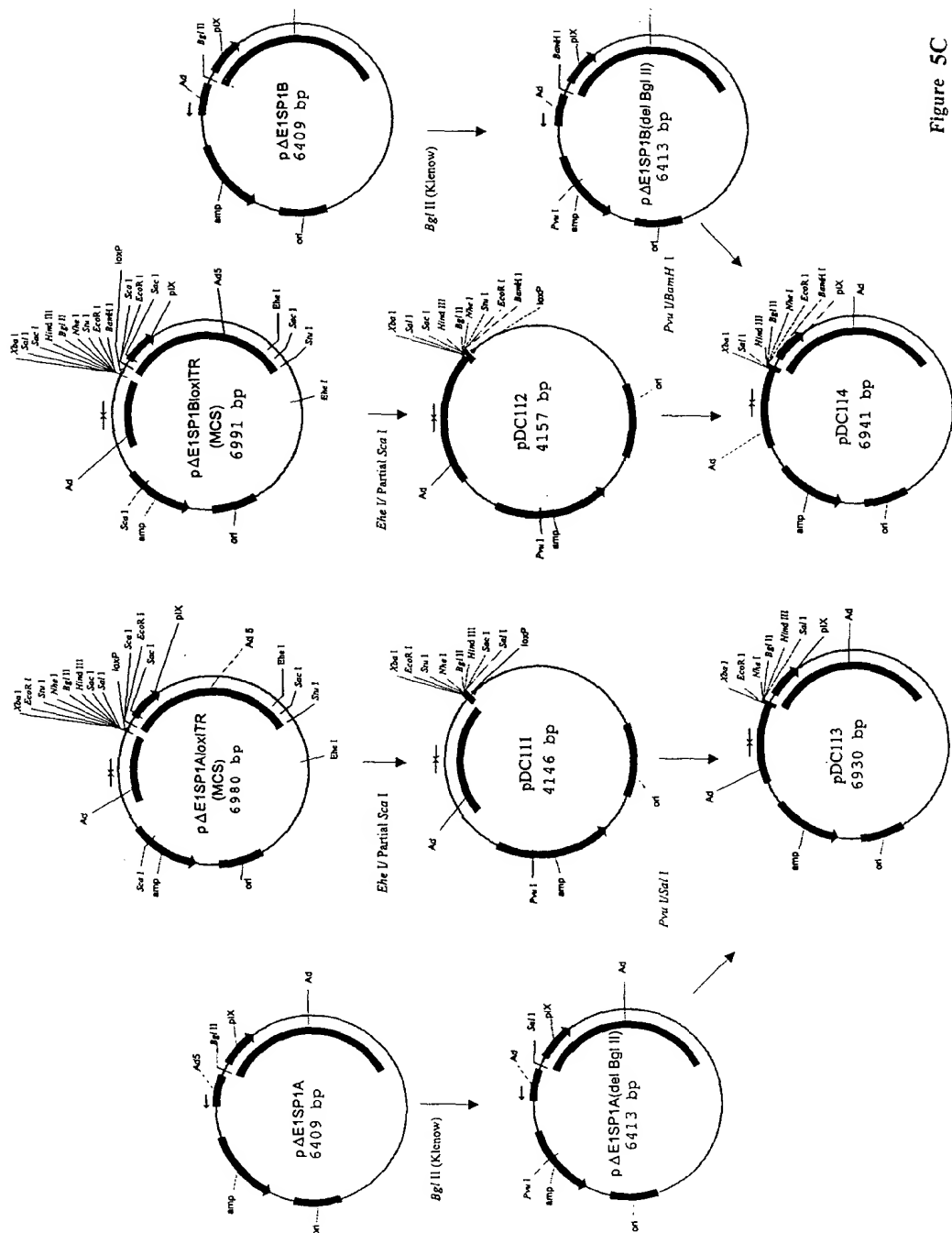
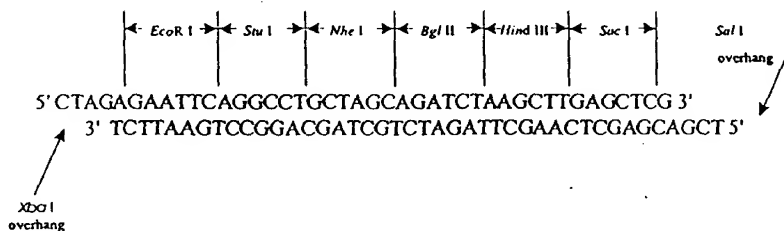


Figure 5C

097043-193760

SEQ. ID. NO.: 12 (AB16853) and SEQ. ID. NO.: 13 (AB16854)



SEQ. ID. NO.: 14 (AB16855) and SEQ. ID. NO.: 15 (AB16856)

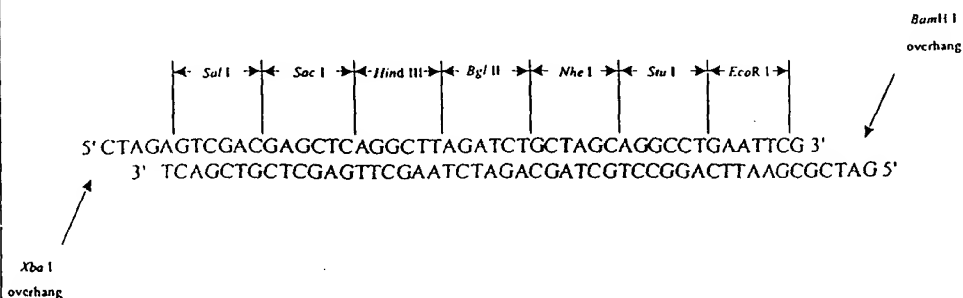


Figure 5D

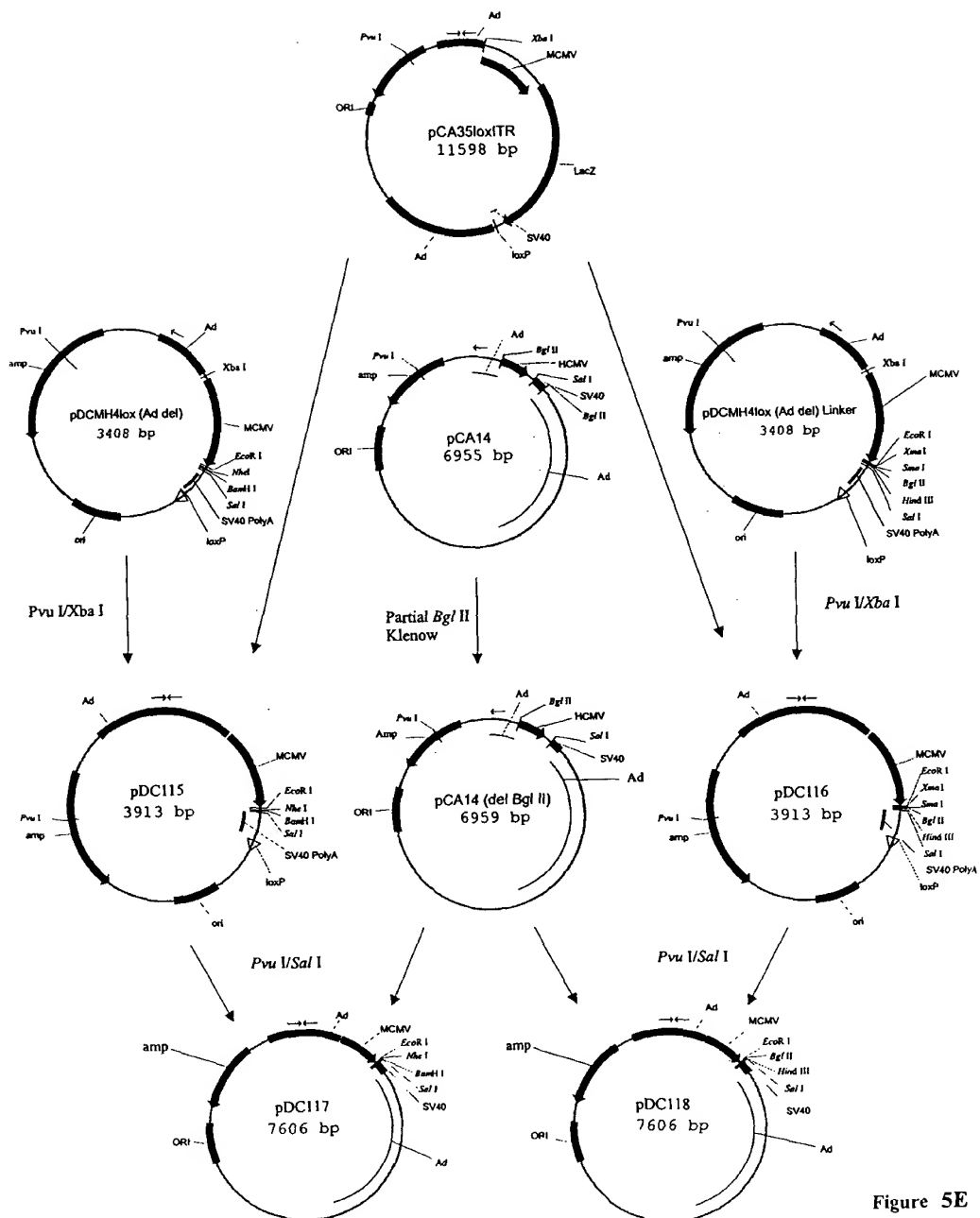


Figure 5E

CONSTRUCTION OF pMH4LOX, pMH4LOX Δ and pMH4LOX Δ LINK SHUTTLE PLASMIDS FOR RESCUE OF EXPRESSION CASSETTES

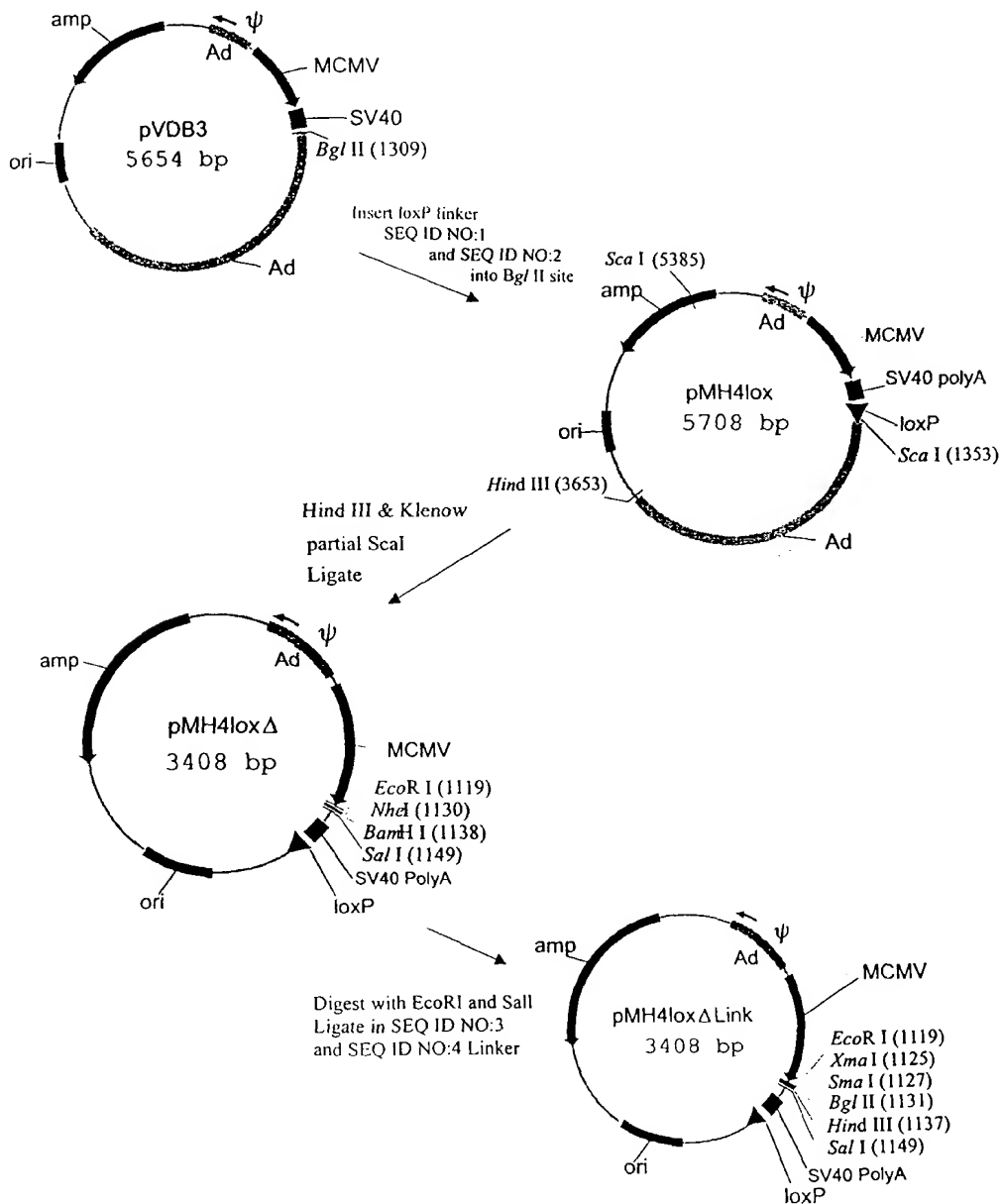


Fig. 6A

CONSTRUCTION OF A SHUTTLE PLASMID CONTAINING A pUC DERIVED ORIGIN

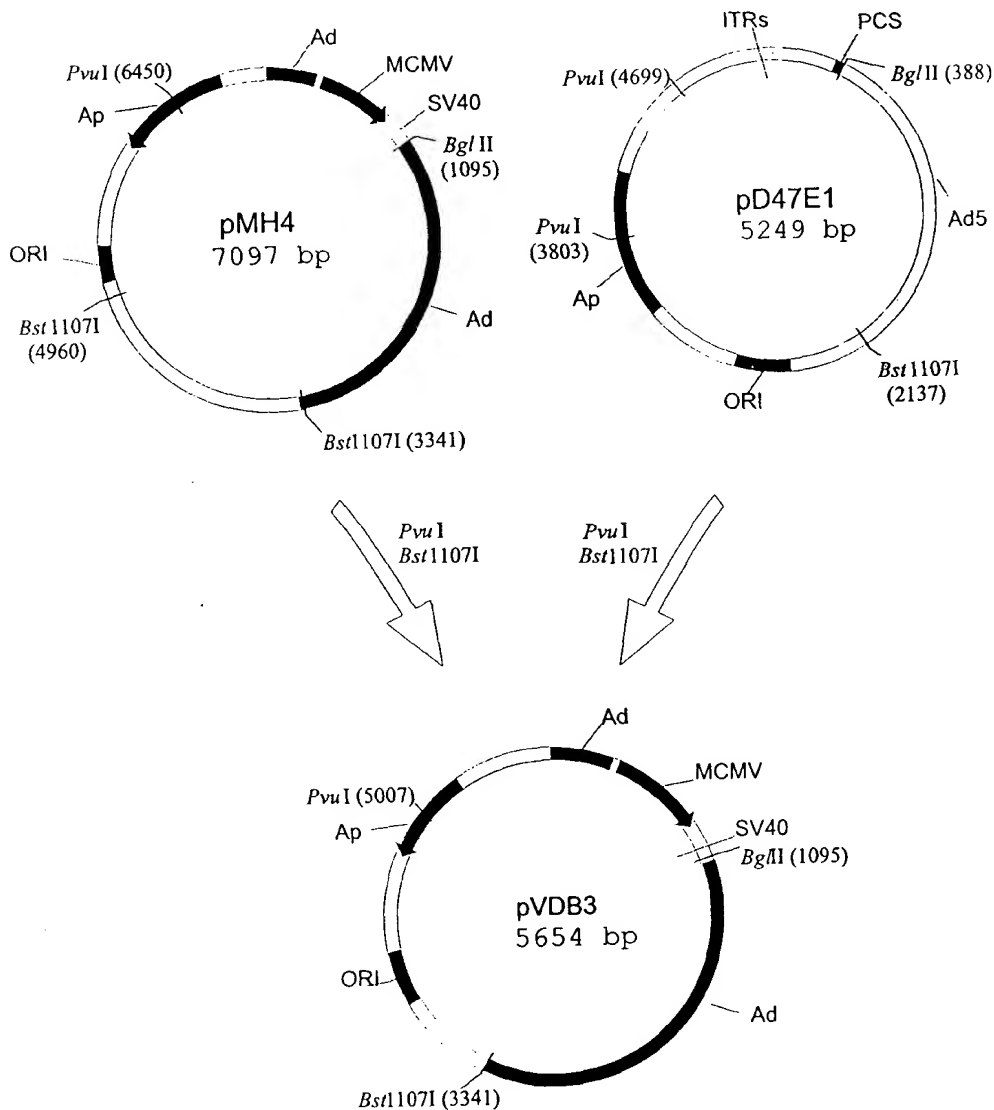


Fig. 6B

CONSTRUCTION OF HCMV loxP PLASMIDS FOR RESCUE OF EXPRESSION CASSETTES

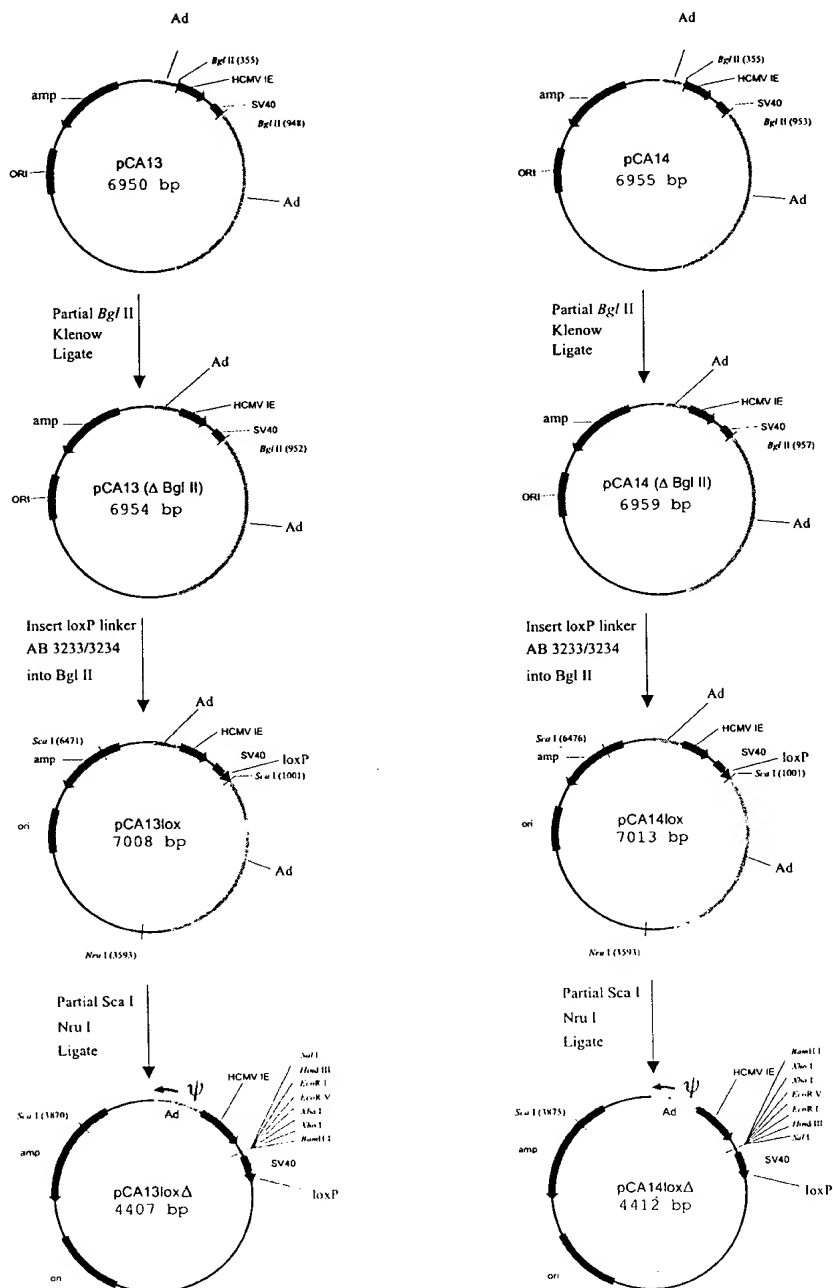


Fig. 7

CONSTRUCTION OF pCA36LOX and pCA36LOX Δ SHUTTLE PLASMIDS FOR RESCUE OF LACZ

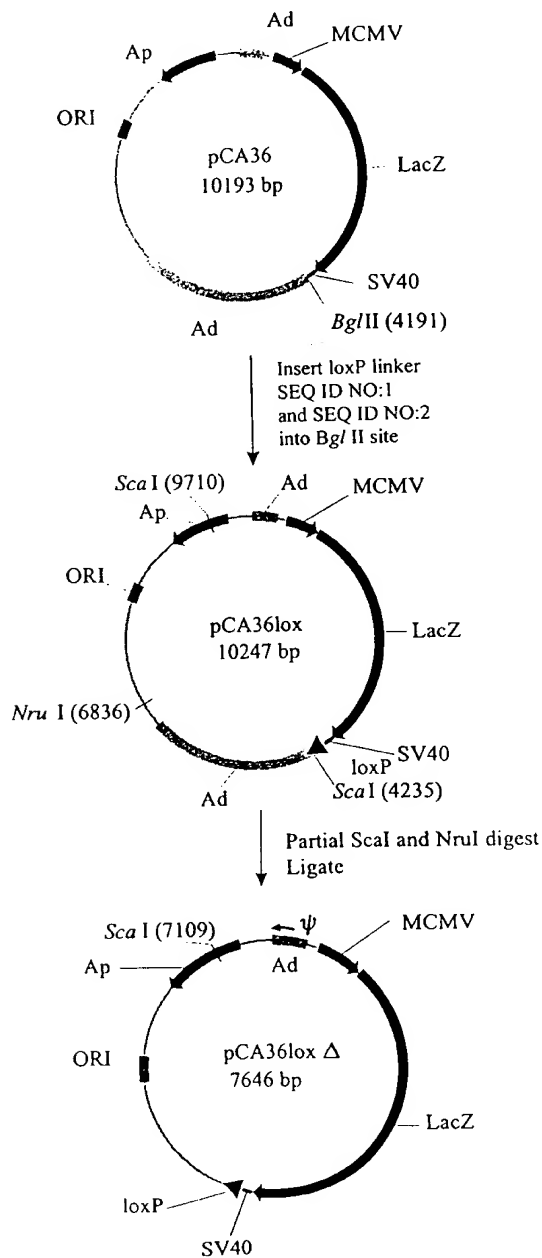


Fig. 8A

Cotransfection of 293Cre cells with AdLC8c DNA-TP and a shuttle plasmid containing a loxP site for generation of Ad expression vectors

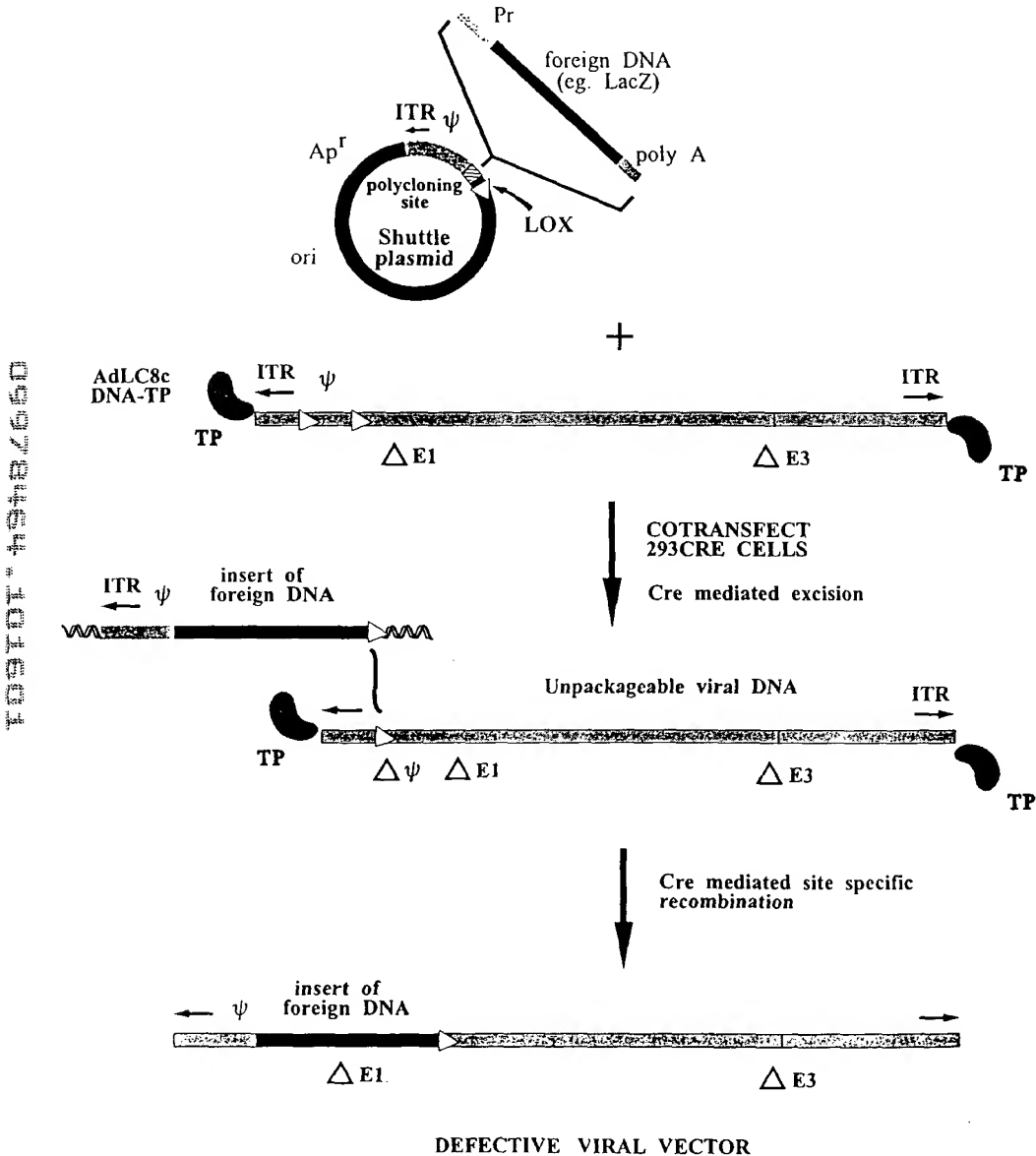


Fig. 8B

Cotransfection of 293Cre cells with restricted AdLC8c DNA-TP and loxP shuttle plasmid for generation of Ad expression vectors

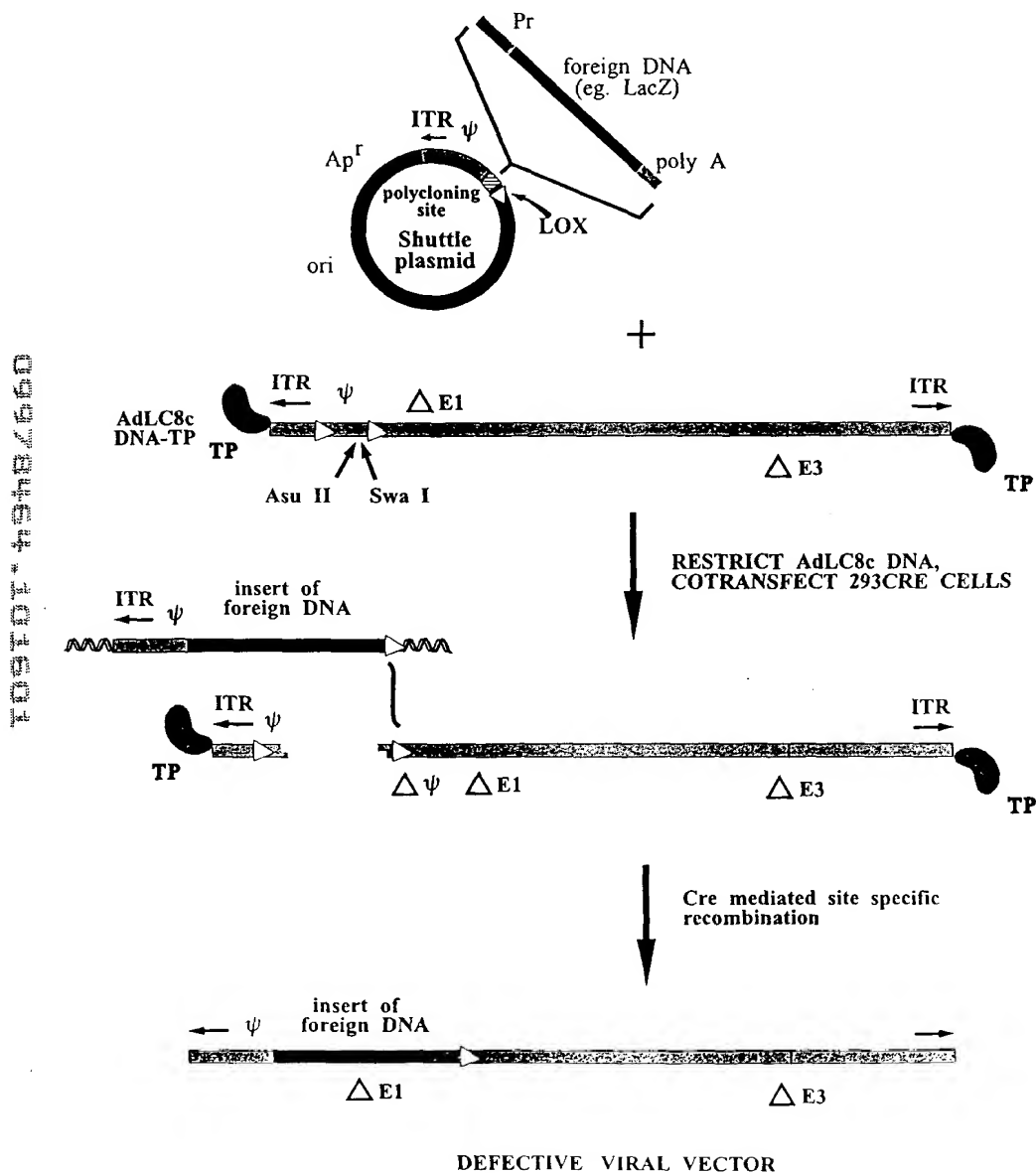


Fig. 8C

CONSTRUCTION OF SHUTTLE PLASMIDS EXPRESSING Cre

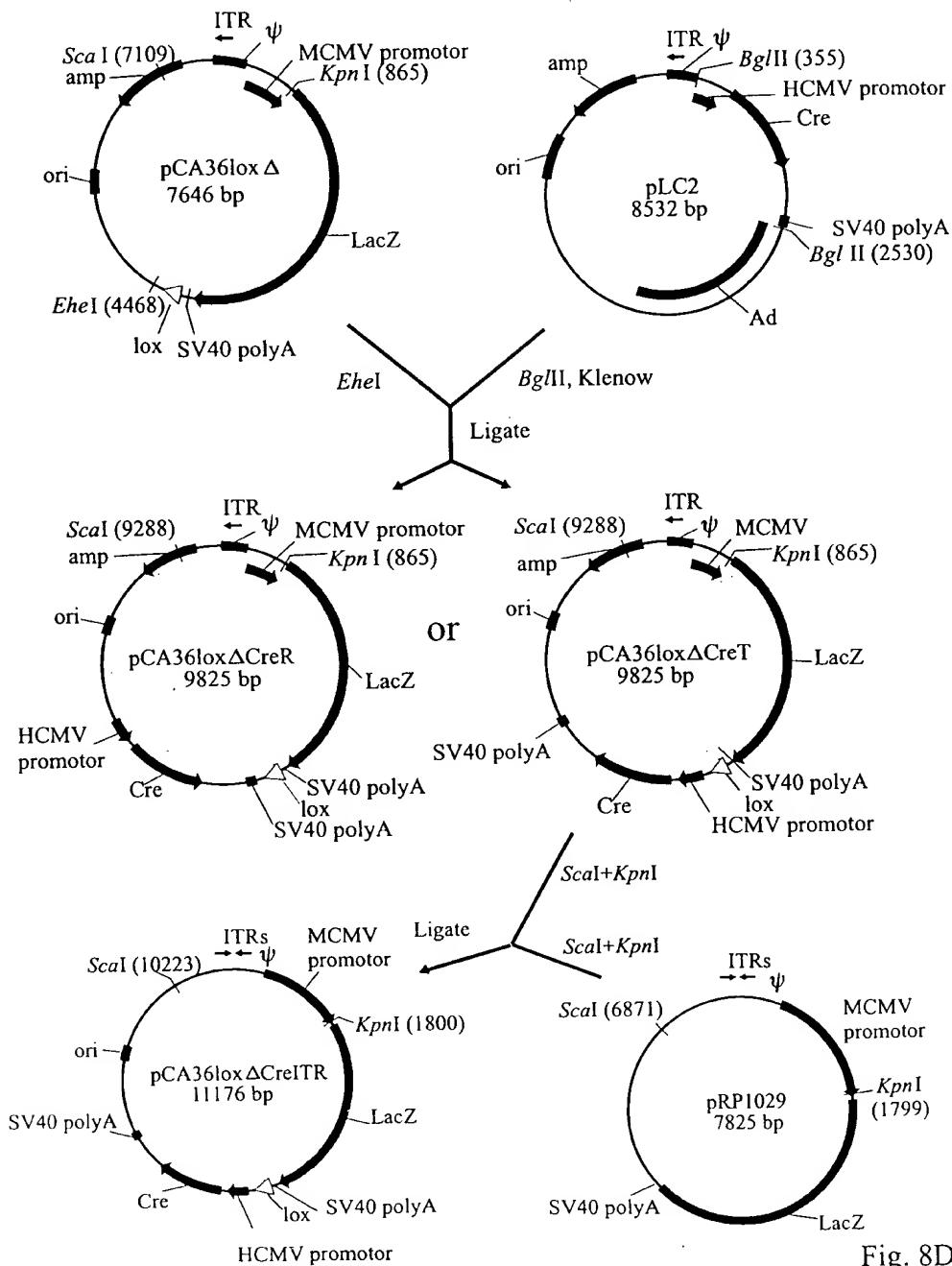


Fig. 8D

Cotransfection of 293 cells with pBHG10lox and a "Lox" shuttle plasmid expressing Cre for generation of Ad expression vectors

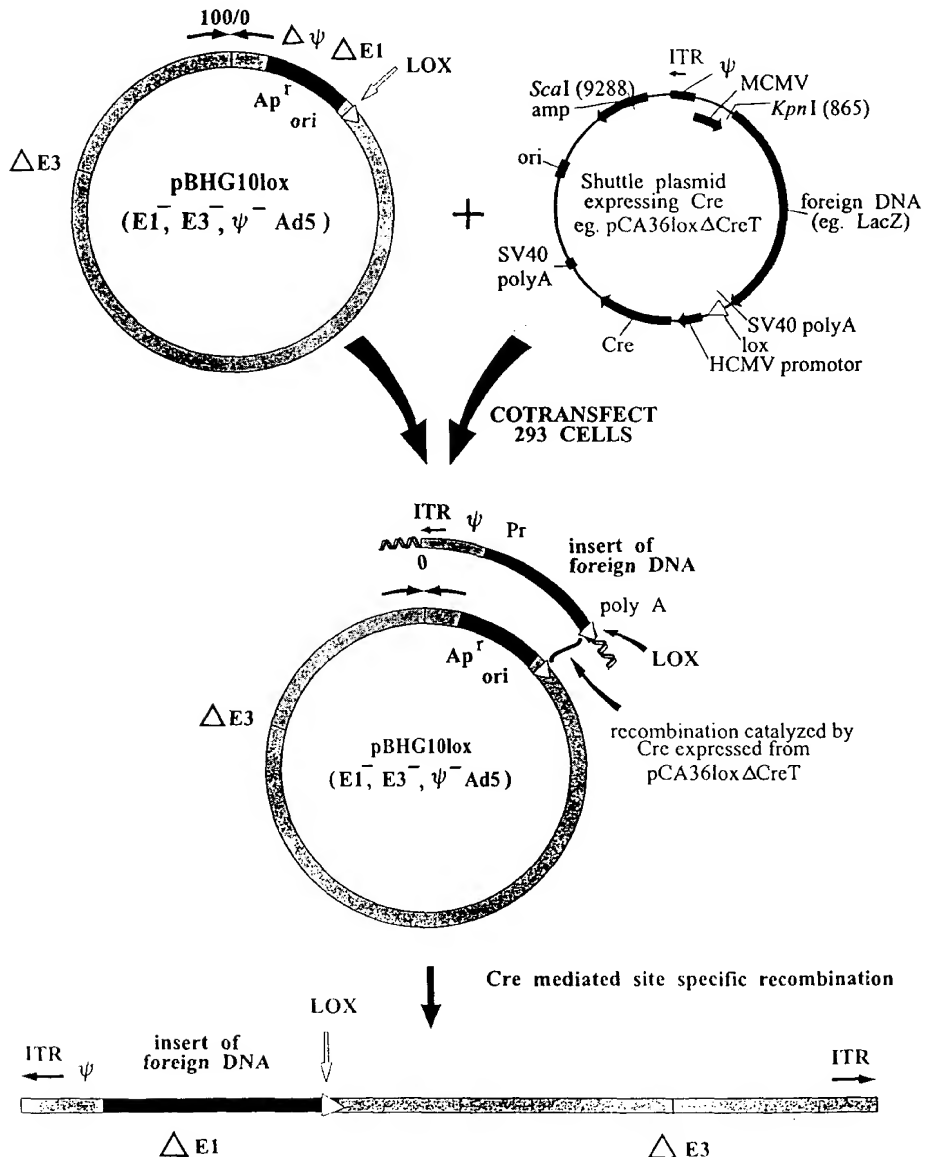


Fig. 8E

CONSTRUCTION OF Ad GENOMIC PLASMID ENCODING CRE

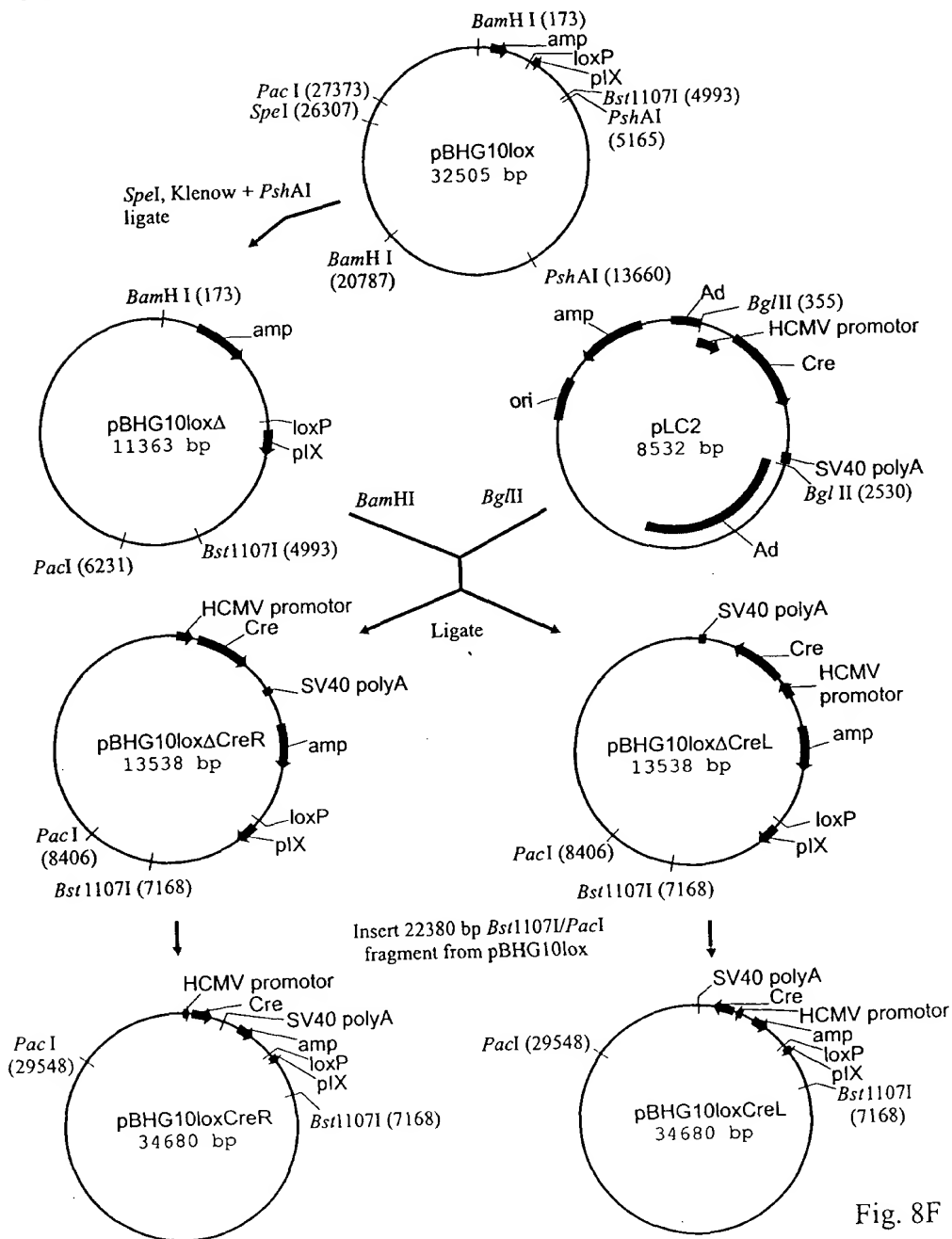
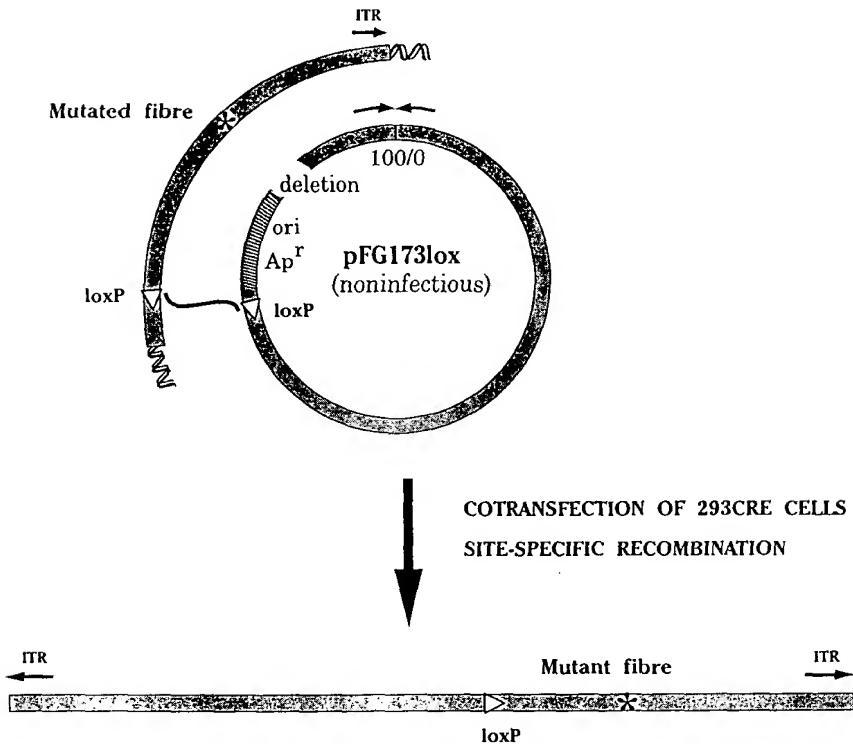


Fig. 8F

RESCUE OF FIBRE MUTATIONS USING CRE/LOX RECOMBINATION



NONDEFECTIVE (E1⁺) VIRUS WITH MUTATED FIBRE GENE

Fig. 9A

CONSTRUCTION OF pAB14lox Δ

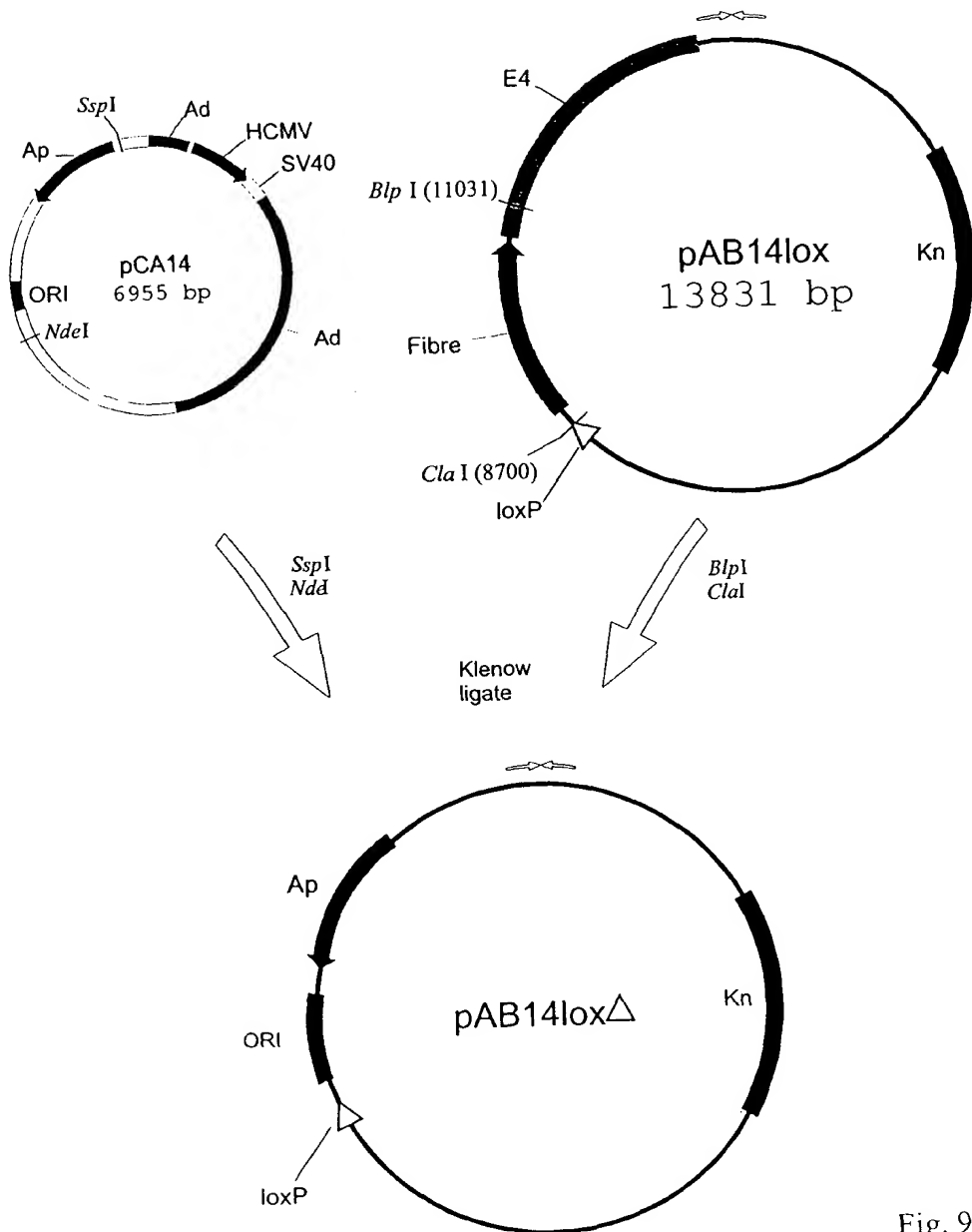
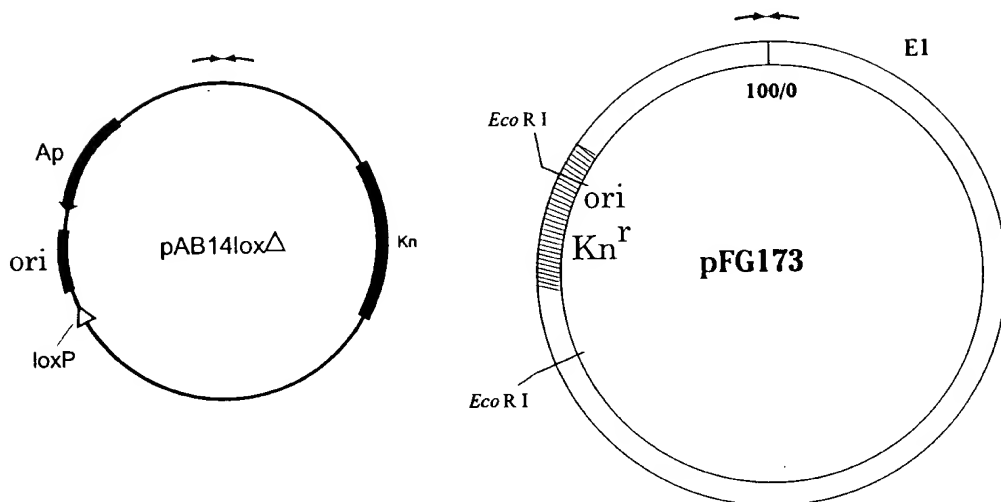


Fig. 9B

CONSTRUCTION OF pFG173lox



Restriction, transformation of *E. coli*,
homologous recombination

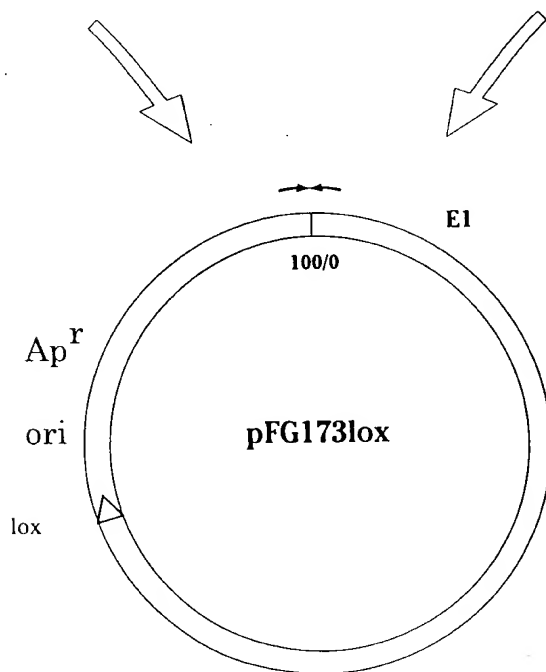


Fig. 9C

CONSTRUCTION OF pFG23dX1lox AND pFG23dX1loxc FOR RESCUE OF MUTANT FIBRE INTO AD VIRUS

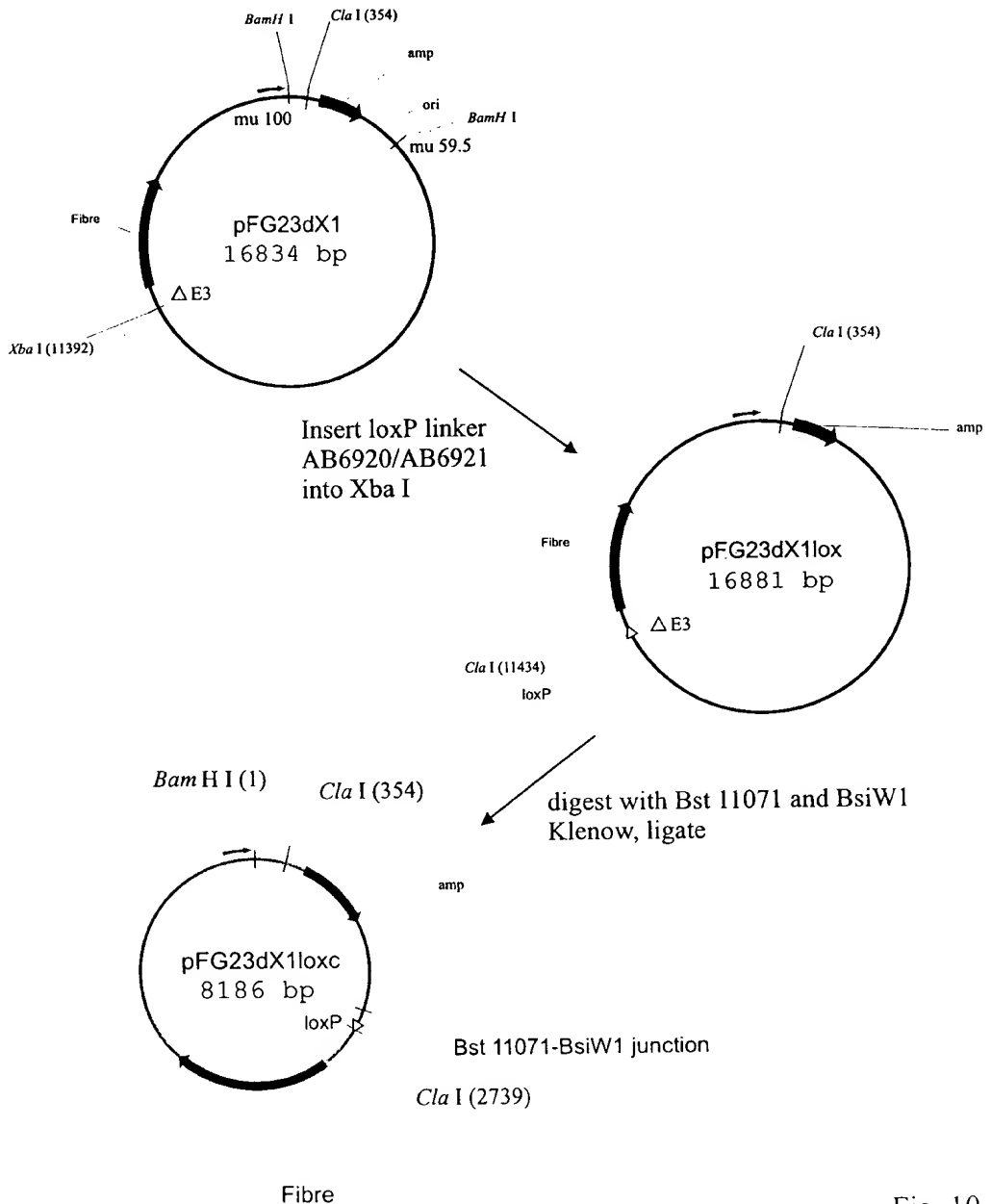


Fig. 10

A PLASMID FOR RESCUE OF A FOREIGN DNA INTO AD VIRUS

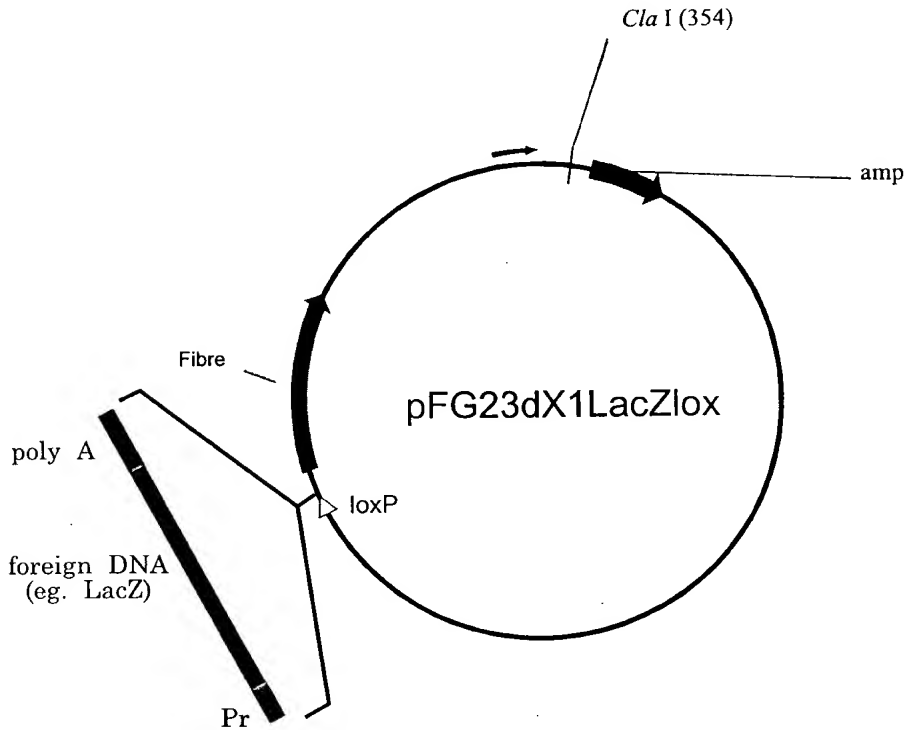
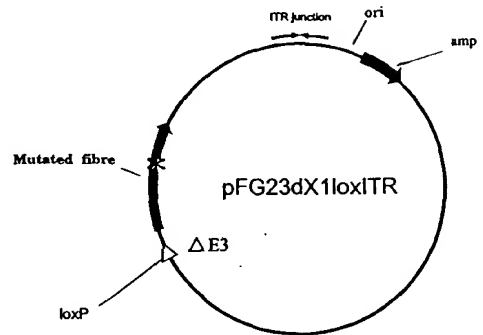
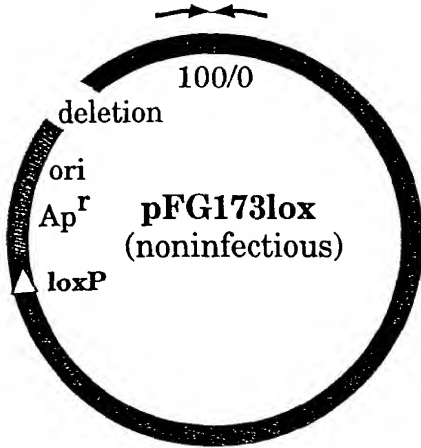


Fig. 11A

RESCUE OF FIBRE MUTATIONS USING CRE/LOX RECOMBINATION



COTRANSFECTION OF 293CRE CELLS
SITE-SPECIFIC RECOMBINATION



NONDEFECTIVE (E1⁺) VIRUS WITH MUTATED FIBRE GENE

FIGURE 11B

Isolation of a virus containing a mutant fibre gene by Cre-lox recombination using DNA-TP and cotransfection

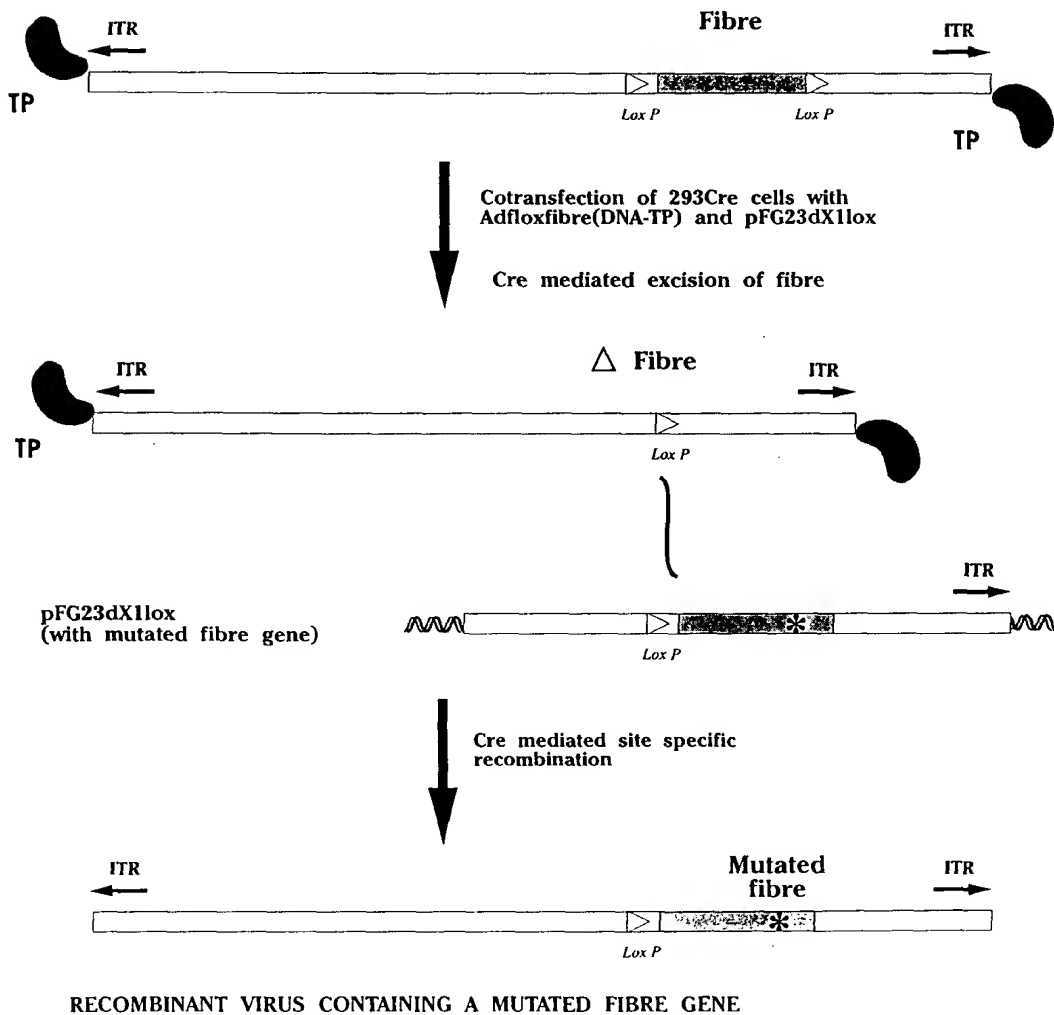


Fig. 12

Isolation of a virus containing a foreign DNA insert upstream of the fibre gene by Cre-lox recombination

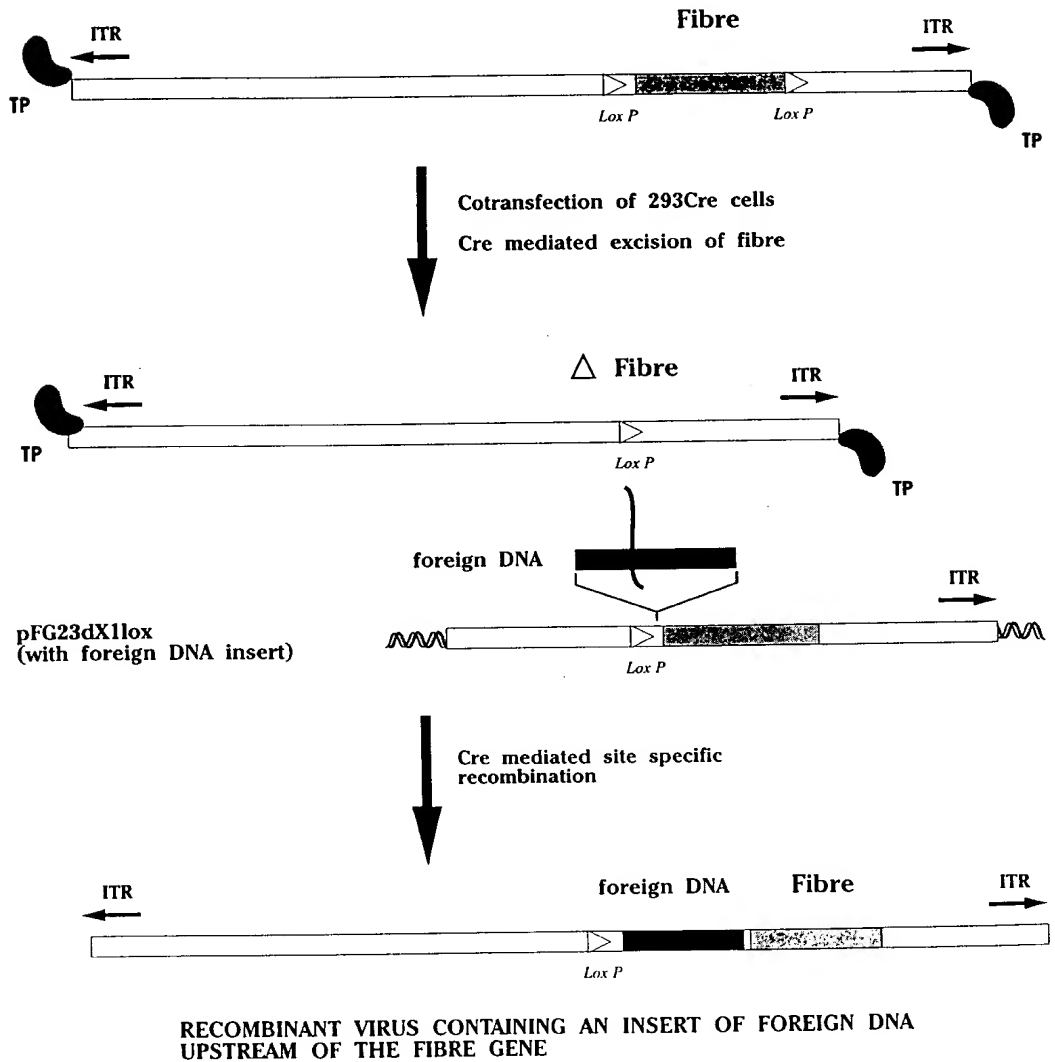
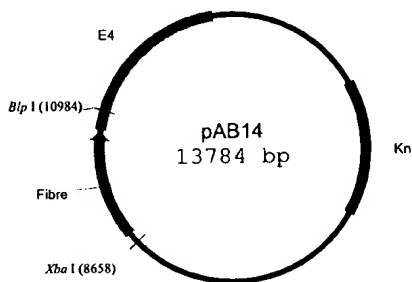
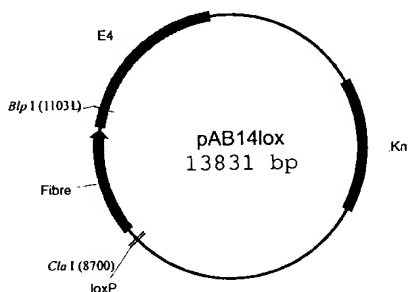


Fig. 13

CONSTRUCTION OF pAB14FL0X FOR ISOLATION OF AN AD VIRUS WITH A FLOXED FIBRE GENE



Insert loxP linker
AB6920/AB6921
into Xba I site



Insert loxP linker
AB14680/AB14681
into Bln I site

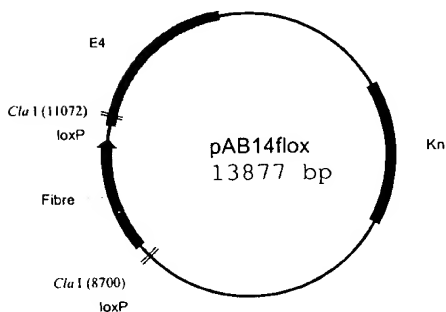


Fig. 14

Isolation of a virus containing a fibre gene with flanking lox P sites.

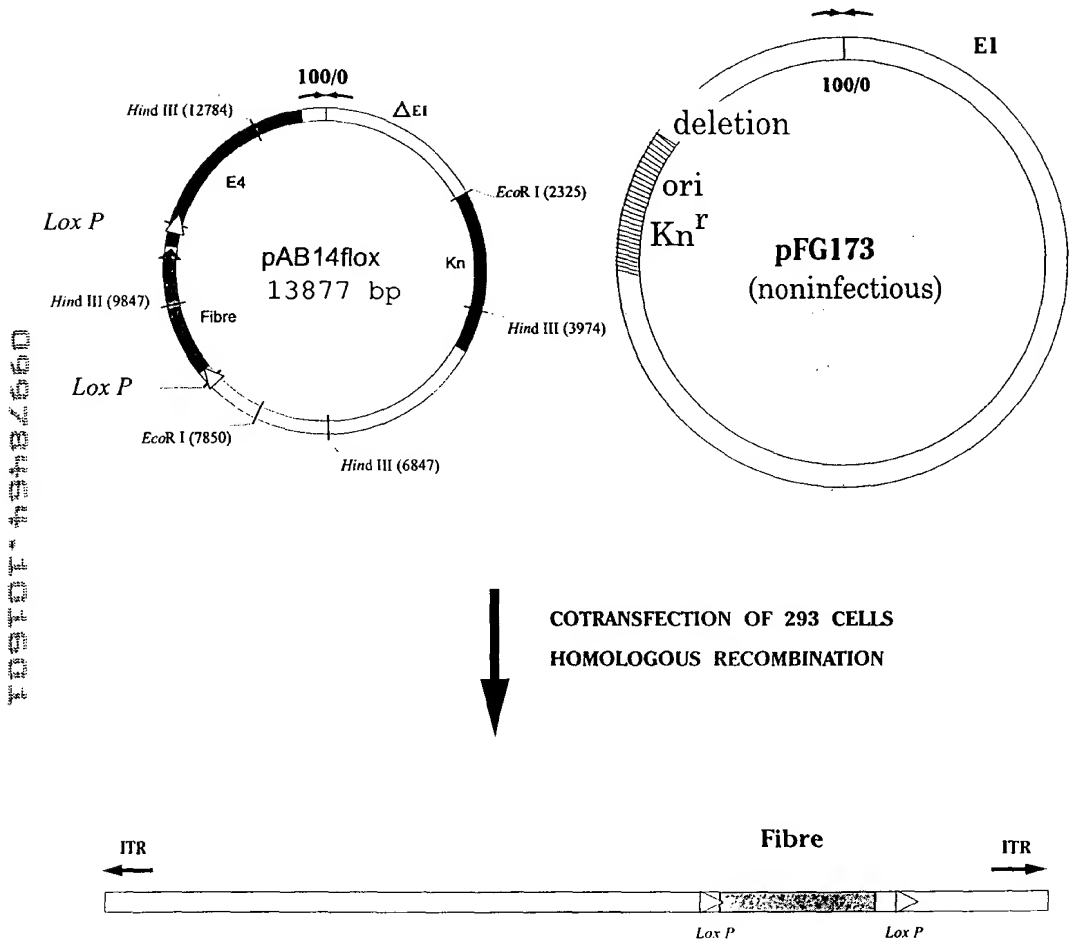


Fig.15